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BULLETIN OF THE MARINE BIOLOGICAL STATION OF ASAMUSHI,
VOL. IX, NO. 2. 1958

HATCHING ENZYME OF THE SEA URCHINS, *HEMICENTROTUS PULCHERRIMUS* AND *HELIOCIDARIS CRASSISPINA*

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The sea urchin eggs were permitted to develop at 15°C. The hatching enzyme begins to be produced within the blastulae, at least from three hours before hatching, and secreted from them into the surrounding medium. Evidence was presented that the membrane-dissolving power is found in the granular fraction, and, as the embryos develop, it is released from the granules into the soluble fraction. These facts proved in *H. crassispina*, were also found in *H. pulcherrimus*, but the activity of the hatching enzyme within the blastulae of *H. crassispina* was weaker than that of *H. pulcherrimus*.

The hatching enzyme of the two species was obtained in crystalline state, but the method for purification was not the same between them, perhaps due to the difference in enzyme proteins.

Although crystalline hatching enzymes of the two species had nearly the same proteolytic activity and almost the same activity dissolving the homologous fertilization membrane, and the ratio of the membrane-dissolving activity to the proteolytic activity of the original hatching enzyme solution did not change during purification of enzyme in the case of *H. crassispina*, on the contrary, in the case of *H. pulcherrimus* the ratio became lower, that is, the proteolytic activity increased about 20 times but the membrane dissolving power only about four times. Two kinds of activator protein were obtained during purification, from the hatching enzyme of *H. pulcherrimus* blastulae. The addition of these two proteins to the crystalline hatching enzyme caused acceleration of the membrane-dissolving power respectively, but gave no effect on the proteolytic activity. These activator protein could not be obtained from the hatching enzyme of *H. crassispina* blastulae. This is the reason why the proteolytic powers could not be separated from each other in *H. crassispina*.

The crystalline hatching enzyme of *H. crassispina* may be expected to contain one or more activator protein, but the sedimentation experiment with the aid of a spinco ultracentrifuge showed clearly a single peak indicating the presence of a single protein.

The hatching enzyme of *H. crassispina* was unable to easily dissolve the

fertilization membrane of *Clypeaster japonicus* and *Mespilia globulus*. It is highly probable that there is some specificity among the sea urchin species.